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Regulation of acute phase reaction in rat adjuvant arthritis

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Introduction

Almost all acute phase proteins are synthesized in the liver and the stimulation of acute phase protein synthesis in liver cells is said to be caused by interleukin-1 (IL-1) derived from macrophages of the inflamed tissue [1]. It is not yet clear if the acute phase protein synthesis is uniformly induced by only one factor released from the inflamed site, or by various agents. Most results concerning these problems have been found *in vitro*. We tested this question *in vivo* during the primary phase of rat adjuvant arthritis by using local low dose administration of the RNA/protein synthesis inhibitor actinomycin D.

Materials and methods

Female rats of a Wistar outbred strain (Falcke, Barby), b.w. 150–160 g, were used. Adjuvant arthritis was induced by injecting 0.1 ml of complete Freund's adjuvant (Institut für Impfstoffe, Dessau) subplantarly into the left hindpaw. Acute phase proteins were determined as described elsewhere [2]. Compounds used were: actinomycin D (Reanal, Budapest), dexamethasone (Organon, Oss), RU 38486 (antiglucocorticoid, ROUSSEL UCLAF, Romainville; kindly supplied by Dr. R. Deraedt and Dr. D. Philibert).

Results and discussion

The results are summarized in the Table 1. Accordingly, in primary adjuvant arthritis the increase of alpha$_2$-macroglobulin serum concentrations, the most prominent acute phase protein in rats, was significantly inhibited by injecting 2.5 or 5 µg actinomycin D into the inflamed paw. The actinomycin D doses of 16 to 32 µg/kg do not influence alpha$_2$-macroglobulin synthesis of the liver in adjuvant arthritis after i.v. or i.m. injection (data not shown). Therefore, the present data might refer to an inhibited synthesis of inflamed tissue derived factors, e.g. IL-1, inducing acute phase protein synthesis in the liver although the initial IL-1 release by macrophages has been found to be independent of new protein synthesis [3]. The alpha$_2$-macroglobulin level was reduced despite an increased paw swelling after 5 µg actinomycin D (Table 1). Although actinomycin D also produced a lasting moderate paw edema in healthy rats it caused no acute phase reaction (data not shown). This finding confirms the above view of an inhibited IL-1 synthesis at the inflamed site.

The increase of caeruloplasmin serum activity was not inhibited by local actinomycin D (Table 1). This could mean that the inflamed tissue factor triggering the increase of caeruloplasmin blood levels does not depend on de novo protein synthesis. This finding agrees with the view that there might be several factors derived from the inflamed tissue which induce acute phase protein synthesis, not only IL-1 [4].

Glucocorticoids together with catecholamines are apparently involved in the regulation of acute phase protein synthesis [5]. The effect of

Table 1
Influence on acute phase protein serum concentrations and on paw swelling in primary adjuvant arthritis.

Group	PS ml	alpha$_2$-MG mg/ml	Cp absorbance	Al mg/ml
1st Experiment				
Control n.a.	0	0.12±0.03	0.21±0.02	36.2±2.6
Control a.	0.43±0.12	2.74±0.97	0.40±0.02	18.1±2.6
ACT D 2.5 µg a.	0.48±0.11	0.91±0.47[b]	0.42±0.08	20.8±1.7[b]
DEX 0.1 mg/kg a.	0.25±0.07[b]	9.93±1.83[b]	0.31±0.06[b]	25.2±0.3[b]
DEX+ACT D a.	0.36±0.05[c]	5.23±1.45[c]	0.25±0.06	24.6±2.7
2nd Experiment				
Control a.	0.44±0.09	0.83±0.24	0.60±0.01	18.6±2.1
ACT D 5 µg a.	0.70±0.13[b]	0.53±0.19[b]	0.66±0.09	18.0±3.7
DEX 0.2 mg/kg a.	0.21±0.07[b]	12.10±2.90[b]	0.43±0.08[b]	24.9±3.6[b]
DEX+ACT D a.	0.30±0.05[c]	6.90±1.20[c]	0.38±0.03	25.7±2.1
3rd Experiment				
Control a.	0.62±0.02	1.52±1.09	0.53±0.12	16.1±3.1
RU 486 10 mg/kg a.	0.56±0.15	0.66±0.21[b]	0.57±0.11	20.1±2.4[b]
DEX 0.1 mg/kg a.	0.24±0.05[b]	7.50±1.64[b]	0.31±0.04[b]	28.4±2.9[b]
DEX+RU486 a.	0.32±0.07[c]	2.26±0.78[c]	0.43±0.10[c]	21.6±1.2[c]

n.a. = non-arthritic; a. = arthritic; PS = paw swelling; MG = macroglobulin; Cp = caeruloplasmin; Al = albumin.
[b, c] Significant difference ($p < 0.05$; t-test) versus arthritic control and dexamethasone group, respectively; only these significant differences are given. Single subplantar injection of actinomycin D as an aqueous solution into the left hindpaw immediately before adjuvant injection on day 1. Oral administration of dexamethasone at days 1 and 2, once daily in experiments 1 and 2; p.o. medication of dexamethasone and RU38486 at days 1–3 in experiment no. 3. Sampling of blood from the orbital vein plexus for acute phase protein determination and measurement of paw swelling 18–22 hours after the last drug administration. n = 8 per group.

dexamethasone on alpha$_2$-macroglobulin levels was significantly reduced by local actinomycin D contrary to the glucocorticoid action on albumin and caeruloplasmin levels which remained unaffected (Table 1). This might indicate a partial connection between the dexamethasone action on acute phase protein synthesis and inflamed tissue, e.g. via a positive feedback. All effects of 0.1 mg/kg dexamethasone (p.o.) on acute phase protein levels and on paw swelling in primary adjuvant arthritis were found to be significantly inhibited by simultaneous oral administration of 10 mg/kg of the potent glucocorticoid antagonist RU38486 (Table 1). The effects on acute phase protein levels and on paw swelling, respectively, after administration of the antiglucocorticoid alone (Table 1) possibly indicate a certain role of endogenous glucocorticoids in regulating the synthesis of alpha$_2$-macroglobulin and albumin but not in modulating the degree of macroscopic inflammation.

Conclusion

Local low dose injection of actinomycin D into the inflamed tissue as well as administration of a glucocorticoid antagonist is apparently suitable for investigating the regulation of acute phase reaction *in vivo*. The acute phase protein synthesis in the liver is apparently triggered by several factors derived from the inflamed tissue, not only by one substance (IL-1).

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